via Smad3- and Smad4-dependent pathways in synergy with bHLH transcription factors such as TFE3. However, despite the presence of two Smad-binding elements and an E-box at the 4G / 5G polymorphism in the PAI-1 promoter, this region does not appear to be a major contributor to TGF-β induction of PAI-1 promoter activity. Taken together, these data indicate that TGF-β is unlikely to be an allele-selective regulator of PAI-1 gene expression.

10:48 a.m.

1007MP-130 Thrombin Activatable Fibrinolysis Inhibitor (TAFI) Levels in Patients With Coronary Artery Disease Investigated by Angiography

Tushar Chatterjee, Verena Schroeder, Martin Fleisch, Stefan Windecker, Christian Seiler, Franz R. Eberli, Bernhard Meier, Hans P. Kohler, *Swiss Cardiovascular Center Bern, University Hospital, Inselspital, Bern, Switzerland, Laboratory for Thrombosis Research, University Hospital, Inselspital, Bern, Switzerland.*

Background: Thrombin activatable fibrinolysis inhibitor (TAFI), also known as plasma procarboxypeptidase B or carboxypeptidase U, is a recently described component which is involved in the regulation of the balance between coagulation and fibrinolysis. When activated by the thrombin-thrombomodulin complex, TAFI leads to a potent inhibition of tPA-induced fibrinolysis. High TAFI antigen plasma levels may therefore contribute to an increased risk for thrombotic disorders. There are no data available about TAFI antigen levels in patients with coronary artery disease (CAD) and subjects free of CAD both investigated by angiography.
Material and Methods: 123 subjects admitted for angiography for investigation of CAD were studied. 81 subjects had 1, 2 or 3 vessel disease, 42 subjects were free of CAD and used as controls. TAFI antigen levels were analysed in citrated blood samples which were taken from the ostium of the left coronary artery. TAFI antigen levels were determined with a commercially available sandwich ELISA (Kordia, Leiden, The Netherlands).
Results: TAFI antigen presented a large range of values, with a 2- to 3-fold increase between the 10th and 90th percentiles in patients and controls (mean: 129.3%). CAD patients had higher plasma TAFI antigen levels compared to controls. (135,25 % vs 114.2 %; p=0.01). TAFI antigen levels remained significant between the groups after adjustment for cholesterol, triglyceride, gender and smoking (p<0.05).
Conclusion: These findings suggest that increased TAFI antigen levels determined in arterial coronary blood may represent a risk factor for CAD. Because of the large interindividual variability of TAFI antigen levels in plasma, genetic control may be involved. Further studies are required to evaluate the significance of the observed changes in TAFI antigen levels in relation to the development of CAD.

POSTER SESSION

1008 Growth Factors, Progenitor Cells, and Angiogenesis

Sunday, March 17, 2002, 9:00 a.m.-11:00 a.m.
Georgia World Congress Center, Hall G
Presentation Hour: 9:00 a.m.-10:00 a.m.

1008-71 Arteriogenesis on Demand in Exercising Mice Expressing FGF-2 Under the Control of the Phosphoglycerate Kinase Promoter

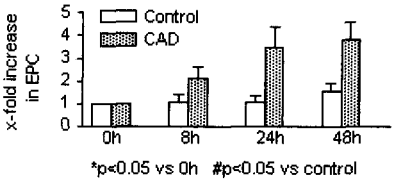
Swen Wolfram, Tibor Ziegelhoeffer, Borja Fernandez, J. Douglas Coffin, Shawn Wagner, Armin Helisch, Wolfgang Schaper, *Max-Planck-Institute, Department of Experimental Cardiology, Bad Nauheim, Germany.*

Background: Fibroblast growth factor 2 (FGF-2), a potent mitogen for endothelial and vascular smooth muscle cell, is implicated in arterial and capillary growth. Exercise is beneficial for patients with peripheral arterial obstructive disease (PAOD) considering calf blood flow, maximal walking distance, and capillary growth. This study investigated the role of regular endurance exercise in a model of PAOD in transgenic mice overexpressing FGF-2.
Methods: The right femoral arteries of 12 transgenic mice overexpressing FGF-2 and 12 nontransgenic littermates (FVB/n) mice were occluded. The mice were randomly assigned to a sedentary group and a group, which was trained 6 days/week on a mouse treadmill. At the end of the exercise program (5 weeks) foot blood flow was determined by Laser Doppler Imaging (LDI). Gastrocnemius blood flow was measured using Magnetic Resonance Imaging (MRI). Exercise capacity of all groups was determined by a graded exercise test. Postmortem angiograms and histomorphometry of collateral arteries were performed.
Results: Only trained FGF-2 transgenic mice demonstrated significantly improved blood flow determined by both LDI and MRI when compared to sedentary nontransgenic mice.
LDI of foot (right/left ratio): 0.97 ± 0.03 vs. 0.66 ± 0.10
MRI of gastrocnemius (ml/min/g) occluded leg: 0.85 ± 0.06 vs. 0.60 ± 0.06
MRI of gastrocnemius (ml/min/g) normal leg: 1.16 ± 0.11 vs. 0.83 ± 0.06
Exercise capacity of trained FGF-2 transgenic animals was dramatically improved (308% of sedentary nontransgenic mice). Postmortem angiograms showed the formation of a dense collateral network in trained FGF-2 transgenic mice. Histomorphometry of collaterals confirmed the angiographic findings of a changed collateral growth pattern in transgenic mice due to exercise.
Conclusion: Trained FGF-2 transgenic mice demonstrate increased blood flow to the foot and to the gastrocnemius, improved exercise capacity, and the formation of a dense collateral artery network. This study indicates that only sufficient FGF-2 application and endurance training lead to improved collateral artery growth. This might be a relevant therapeutical approach for humans suffering from PAOD.

1008-72 Endothelial Progenitor Cell Release in Patients With Coronary Artery Disease After Maximal Symptom-Limited Exercise Test

Karsten Lenk, Volker Adams, Dominik Lenz, Atila Tarnok, Sandra Erbs, Gerhard Schuler, Rainer Hambrecht, *University of Leipzig, Heart Center, Leipzig, Germany.*

Background: Increasing evidence suggests, that endothelial progenitor cells (EPC) play an important role in postnatal neovascularization. As animal studies propose ischemia seems to be one of the main triggers for the release of EPCs from the bone marrow. Until now, however, less is known about the impact of an acute myocardial ischemia on the EPC release. Therefore, the aim of this study was to investigate the release of EPCs in response to a maximal symptom-limited exercise in patients with symptomatic coronary artery disease (CAD).
Methods: In six patients and six healthy controls blood was taken before and 8, 24 and 48 hours after a maximal symptom-limited exercise test. Exercise tests were stopped in patients due to progressive angina pectoris and/or significant ST segment depression at 95,8± 10Watt. Mononuclear cells (MNC) were isolated and cultured. After 4 days adherent EPC were characterized by Di-acLDL uptake and concomitant FITC-lectin binding. Double positive cells were counted by Laser Scanning Cytometer (LSC). The endothelial origin was further documeted by demonstrating the expression of KDR, vWF and VE-cadherin.
Results:



*p<0.05 vs 0h #p<0.05 vs control

Conclusions: These results demonstrate for the first time that in patients with CAD even a very short episode of myocardial ischemia is sufficient to induce a considerable release of EPCs.

1008-73 Chimeric Vascular Endothelial Growth Factor Fusion Protein Selectively Inhibits Flk-1 Endothelial Progenitor Cells

Jonathan M. Hill, Marina V. Backer, Joseph M. Backer, Toren Finkel, *National Institutes of Health, Bethesda, Maryland.*

Endothelial progenitor cells (EPCs) contribute to postnatal vasculogenesis in tumors and potentially regulate atherosclerotic plaque size. Selective destruction of EPCs may therefore have therapeutic benefit in certain clinical settings. We have made use of the fact that EPCs express high levels of the receptor for vascular endothelial growth factor-2 (VEGFR-2/Flk-1). An active fusion protein SLT-VEGF/L, containing the catalytic A-subunit of Shiga-like toxin fused to VEGF121 and a control fusion protein SLT-VEGF/Lci containing a catalytically inactive mutant of SLT A-subunit (Y114S and R170L amino acid substitutions) fused to VEGF121 were constructed, expressed and purified. EPCs were isolated from human peripheral blood mononuclear cells using density gradient centrifugation and seeded on fibronectin-coated plates. An endothelial phenotype of the fibronectin-adherent cells was confirmed by immunohistochemical analysis for Tie-2, CD31 and VEGFR-2, and by ability of these cells to uptake DiI-labeled acetylated LDL. A dose response curve demonstrated that EPC colony formation was inhibited by SLT-VEGF/L with an IC_{50} of 0.5 nM. This inhibitory effect is ~1000-fold more efficacious than angiostatin, another reported inhibitor of EPC activity. In contrast, the catalytically inactive fusion protein SLT-VEGF/Lci did not affect EPC colony formation. Human umbilical vein cells (HUVECs) expressing, 2-5 $x10^4$ VEGFR-2/cell, and porcine aortic endothelial cells (PAE/KDR) engineered to expressed 2-$3x10^5$ VEGFR-2/cell were employed for evaluating the effects of SLT-VEGF/L on endothelial cells with different VEGFR-2 density. HUVECs were relatively insensitive to SLT-VEGF/L at concentrations up to 20 nM while PAE/KDR cells had an IC_{50} below 1 nM. In summary, a chimeric toxin can inhibit EPC activity, cluster and spindle cell formation. This inhibition is a function of high VEGFR-2/Flk-1 expression on these cells. Native endothelial cells are at least 40-fold less sensitive to the effects of the chimeric toxin. These results suggest the potential clinical applicability of this chimeric toxin for the selective ablation of EPC activity.

1008-74 Tissue Factor Inhibition Impairs the Maturation of Neovascularization in Postnatal Angiogenesis

Frederic Mouquet, Katy Didier, Delphine Corseaux, Albin Pourtier, Sylvie Vincent, Chantal Vercamer, Bernard Vandenbunder, Christophe Bauters, Brigitte Jude, Eric Van Belle, *Hopital Cardiologique, Lille, France, Institut de Biologie de Lille, France.*

Tissue factor (TF) has an important role in vasculogenesis and tumoral angiogenesis. However, its role in post-natal non-tumoral angiogenesis is unknown. We investigated TF inhibition effects in a murine model of angiogenesis. Micro-implant loaded with basic Fibroblast Growth Factor (bFGF) were grafted in ears of balb/c mice, inducing a local angiogenesis. TF was inhibited after systemic injection of an active site-inhibited FVII (ASIS, gift from Novo Nordisk). Angiogenic response was accurately quantified by histological techniques. Five mice were grafted with control implants. Seventeen were grafted with implants loaded with 100 ng bFGF; eight received systemic injection of ASIS (5 µg/